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NMR Binding Studies of Monosaccharides to Cholic Acid Hosts

by

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Synthetic molecular receptors have been prepared by linking two cholic acid moieties with p-xylylene diamine, m-xylylene diamine, or diphenyldichlorosilane. In-depth studies of the p-xylyl and m-xylyl hosts binding with n-pentylglucoside have been carried out by means of 600 MHz NMR. Using $CDCl_3$ as solvent, chemical shift changes have been observed for H_3 and H_4 of the glucoside. Data suggest that the glucoside can bind to all cholic acid dimers studied so far, and also to cholic acid monomers used for comparison.

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I. Introduction and background :

"Molecular Recognition" is a field which has been well developed in past decades. In particular, much attention has been focused on molecules which bind with small neutral species. Sugars are one type of the small natural molecules which are of interest because we might carry out reactions on sugars in organic solvents if a lipophilic host can bind with a hydrophilic sugar, rendering it soluble.

Aoyama et. al.(Ref.1) synthesized a macrocyclic multidentate polyhydroxyl compound which was capable of selective extraction of carbohydrates from water into CCl_4 (Fig.1). This resorcinol-dodecanal tetramer apparently binds with water molecules (Fig.2), providing a hydrogen-bonded network which was studied at various temperatures by ^{1}H-NMR (Ref.2). In this case, a pair of OH groups on adjacent benzene rings of the macrocycle are strongly NOE correlated, but the proton exchange in the resulting hydrogen-bond network is very slow.

Figure.1

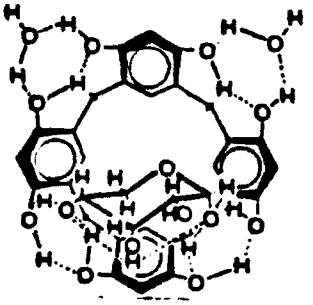
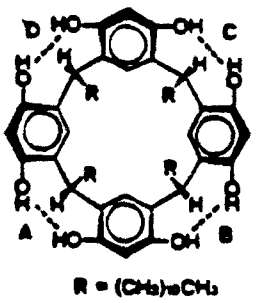
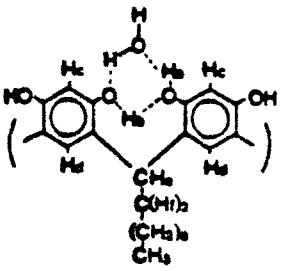


Figure.2





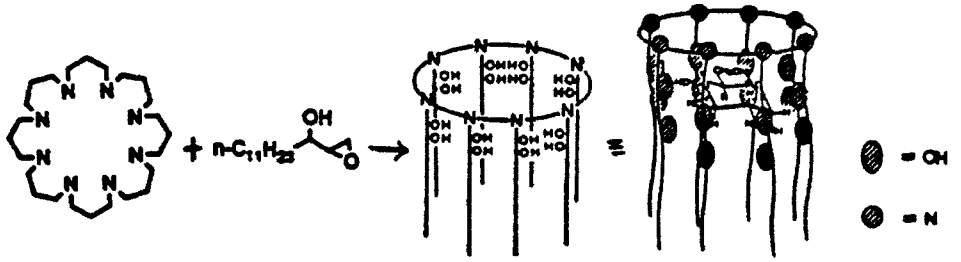
Further, Aoyama reported the highly stereoselective glycosidation of ribose with CH_3OH in CCL_4 via complexation of the resorcinol-dodecanal tetramer and ribose (Ref.3). The ratio of 1:10 for tetramer-ribose complex and CH_3OH in CCl_4 results in a 100% conversion of ribose into methyl β-ribofuranoside which could then independently form a complex with the tetramer. They achieved the first example of synthetic reactions of an unprotected sugar in apolar organic media

under mild and neutral reaction conditions.

More recently, Aoyama tried the tetramer in binding with some regio- and stereoisomers of cyclohexanediols by multiple hydrogen bonds (Ref.4).The four independent binding sites could selectively bind with different diols with different affinities. The geometrical requirements for diols depend on the configuration (axial-equatorial > diequatorial) and relative positions (1,4 >> 1,2 > 1,3) of two OH groups. Their work may provide a new strategy to overcome the difficulties in selective protection-deprotection of OH groups by stereoselective molecular recognition.

Most recently, Aoyama utilized an octaalkylated octaazamacrocycle (Fig.3) to extract sugars such as ribose, fructose and glucose from water into CCl_4 via a flexible microsolvation effect (Ref.5).

Figure.3

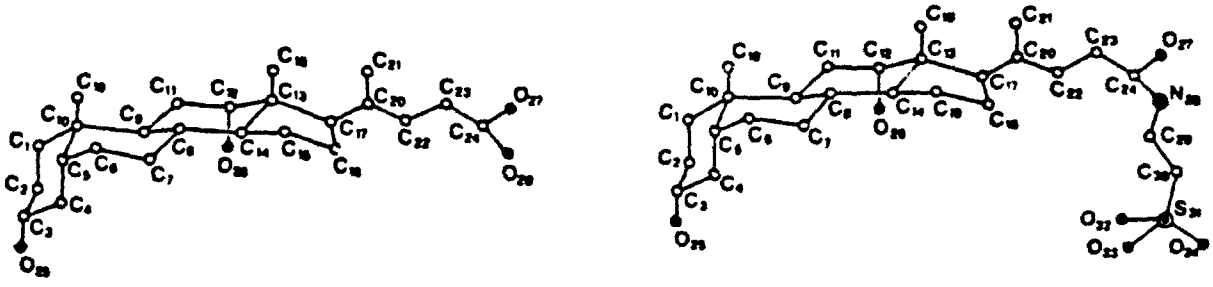


Another example of molecular recognition of saccharides has been developed by Tsakagoshi et. al.(Ref.6), where they used boronic acid derivatives of calixarenes and their acyclic analogs to selectively recognize sugars (e.g. fructose or glucose) through the formation of covalent bonds between $-B(OH)_2$ and diols.

Currently, we are trying to synthesize some receptors to bind with saccharides. Our methodology is to use steroids as the naturally occurring building blocks for our synthetic hosts. Our choice among the steroids is cholic acid, an inexpensive bile acid.

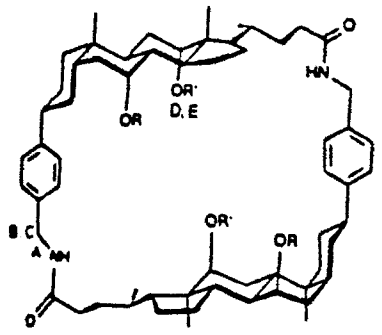
Steroids have been used for molecular recognition both in nature and in synthetic designs. Recently, Giglio et. al.(Ref.7) found that the structure of the micellar aggregates of sodium deoxycholate (NaDC) and sodium taurodeoxycholate (NaTDC) were helical (Fig.4). Those helices are strongly stabilized by ion-ion interactions between cations and carboxylate ions, by ion-dipole interactions between cations and water or hydroxyl groups, and by hydrogen bonds. Giglio also reported that the addition of NaDC or NaTDC caused the breaking of acridine orange(AO) aggregates in aqueous solutions, and the binding geometries of the two bile salts with AO slightly changed.

Figure.4 NaDC NaTDC

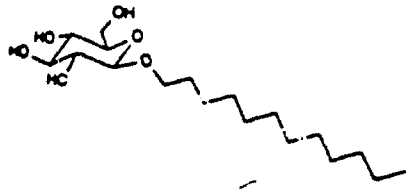


A macrocycle, which is an analog of our target molecules, but possessing a different C_2-axis, has been synthesized by Davis et. al.(Ref.8). Davis used a series of derivatives, called "cyclophanes", to bind with lipophilic glucosides (Ref.9)(Fig.5). By NMR titration studies and calculated binding curves, they found strong binding (Ks $\approx 10^3$ M^{-1}) for the interaction of the cyclophane cavity and n-dodecyl glucoside in $CDCl_3$. They estimated the intermolecular hydrogen bonding was about 2.06-2.32Å and the cavity was about 11.1-14.9Å wide (NH ---- HN distance). Even though they have a macrocyclic compound, the frame of the molecule is very flexible. For example, 36 different conformations have been found within 4.5 Kcal/mol range for compound a. This suggests high flexibility in our target molecules as well.

Figure.5



a: R,R' = H
b: R,R' = Ac
c: R = Ac, R' = Bn
d: R = Ac, R' = H
e: R = H, R' = Bn



Recently, Davis et. al. synthesized another inside-out cyclophane (Ref.10, Fig.6). The compound is a water-soluble cyclophane due to two bulky 3β-substituents. The protected 7&12α-diesters in the cavity were lipophilic and the hydrogens of the amide bonds shirved hydrogen bonding with two molecules of tetrahydrofuran (THF). The X-ray structure showed the cavity was about 13Å wide and N-H···O hydrogen bond was about 3.01Å (Fig.7).

Figure.6

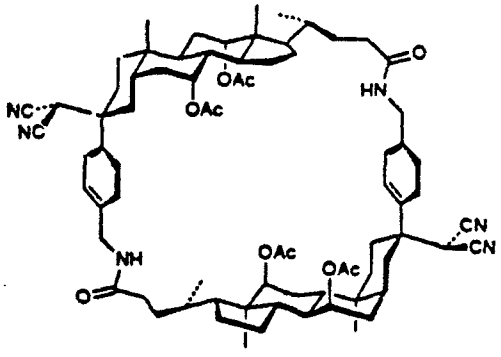
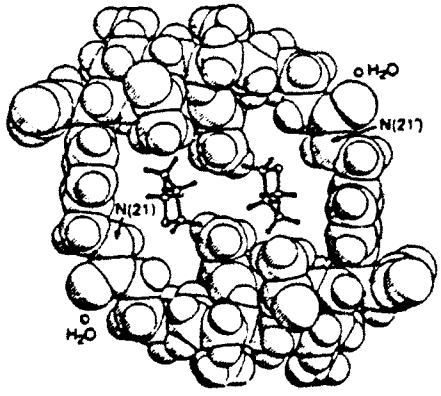


Figure.7



Bonar-Law has synthesized a porphyrin capped with a steroidal superstructure bearing convergent hydroxy groups (Ref.11)(Fig.8). The Zn-porphyrin complex is capable of binding with a variety of functionalized amines via a combination of metal-amine and hydrogen-bonding interactions. The binding affinity was measured to assess the recognition ability of the convergent OH groups. The metal-ligand interaction was used to catalyze and control selective acylation of a single hydroxy group in the cap. Because the Zn-amine interaction is positioning the active

species (presumably an acylpyridinium salt) for rapid intramolecular reaction; only one hydroxy group is acylated to form a "ligand-tailed"compound (Fig.9). Since the Zn atom in the product is pentacoordinate, it showed no tendency to bind another ligand. They synthesized the doubly-capped chiral functionalized porphyrins and demonstrated both cooperative multipoint binding and metallo-porphyrin directed catalysis of an intramolecular reaction.

Figure.8

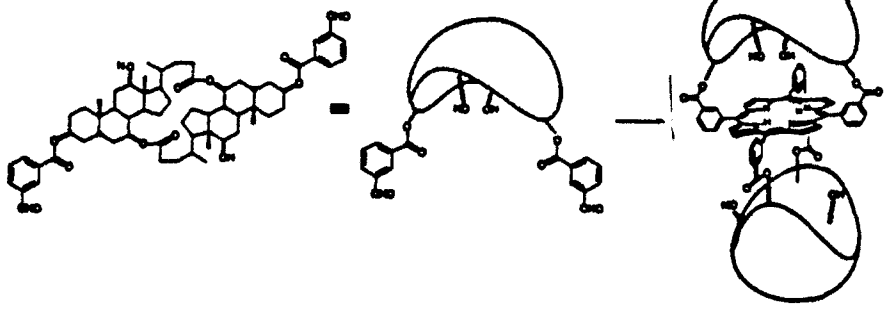
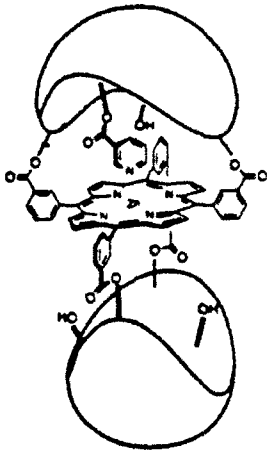
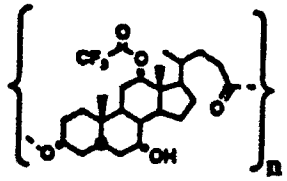


Figure.9

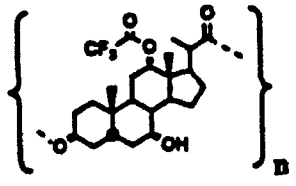


Additionally, Bonar-Law investigated macrocyclic lactones (Fig.10), which are called "cyclocholates"(Ref.12). Cyclocholates are cyclic hydroxylated steroids derived from cholic acid that are bound with polyhydroxylated ligands such as 1,3,5-trihydroxybenzene in organic solutions.An endoreceptor of novel geometry, the "molecular bowl" (Fig.11),has been prepared from a functionalized cyclocholate.

Figure.10



n = 2,3,4,5,6



n = 3,4,5,6

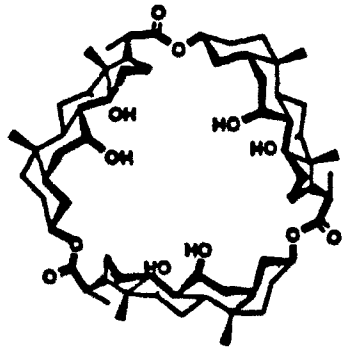
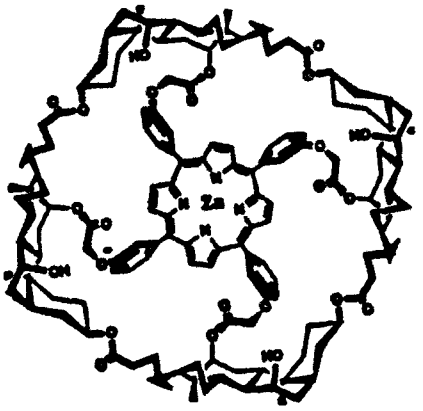


Figure.11



Gokel et. al. have also uesd steroids in host-guest chemistry (Ref.13). They prepared one cholesteryl-ferrocene compound (Fig.12). The neutral molecule exhibited no aggregation phenomena in solution, but the cation, approximately spherical versicles with a membrane of ca. 45Å thickness were found. This is the first example of vesicle formation from hydrophobic ferrocene derivatives. In the following paper (Ref.14), Gokel postulated the two coplanar cyclopentadienyl rings of a ferrocene may rotate with respect to each other about the iron center as if the metal were a ball-bearing between plane surfaces. So it is now possible to design a system which has all of the functionality for binding of a particular molecule, but which does not have functionality in the correct spatial position. The active conformation of receptor is induced by the presence of the substrate to have binding of difunctional molecules to a ferrocene receptor (Ref.15)(Fig.13).

Figure.12

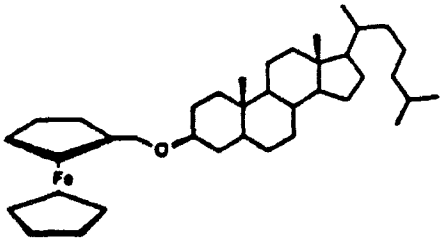
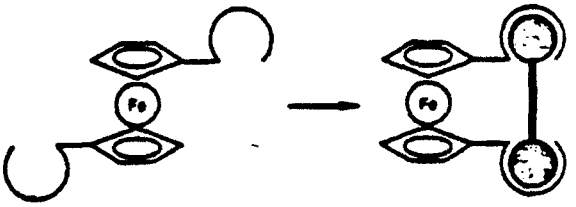


Figure.13



II. Results and discussions:

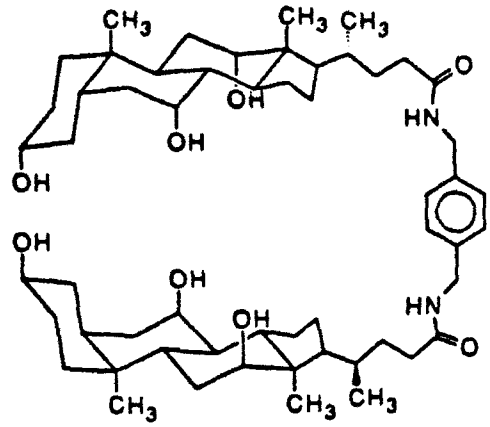
We used cholic acid to synthesize the target molecules listed below:

1. p-cholamide dimer
2. p-bis-nor-cholamide dimer
3. m-cholamide dimer
4. m-bis-nor-cholamide dimer
5. 3&-diphenylsilyl cholate dimer
6. mono-cholamide

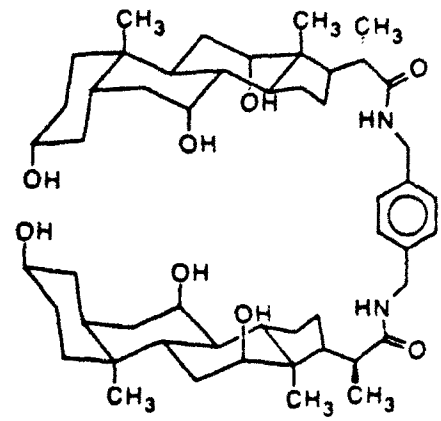
The chemical shift changes for both dimer and glucoside were examined by ^{1}H-NMR at 303°K. In the case of the m-cholamide dimer, the chemical shifts associated with amyl-glucoside were observed to move. The mono-cholamide was used as a control molecule in order to evaluate of what type of binding occurred. The results showed that the control monomer had the same chemical shift changes on associating with the glucoside as did the m-cholamide dimer. The amide hydrogen of monomer had a small downfield shift. This matched calculations of theoretical modelling which will be mentioned in part III. The m-cholamide dimer only had one free cholate arm to bind with glucoside, the other arm was totally twisted which may be due to the intramolecular hydrogen bonding between two amide bonds in the molecule. The control monomer has only one free cholate arm to bind with the glucoside which is the same as the twisted m-cholamide dimer.

Additionally the binding of the p-cholamide dimer was investigated. All peaks of the glucoside with p-cholamide dimer in the range of δ=3-5 ppm behaved as m-cholamide dimer case did. This showed that all of cholamide dimers and monomer could bind with amyl glucoside and have similar binding phenomena. Further informations about the binding constants and ratios will be studied by titration experiments at NMR.

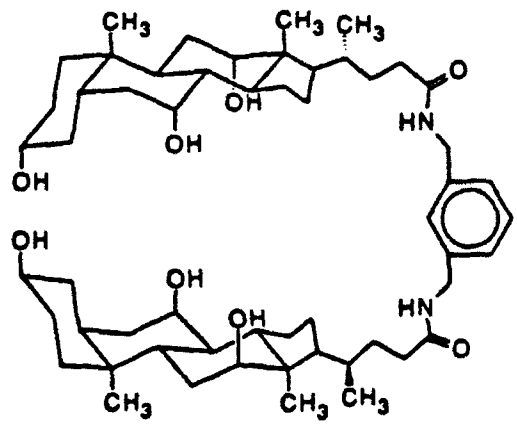
Target molecules :



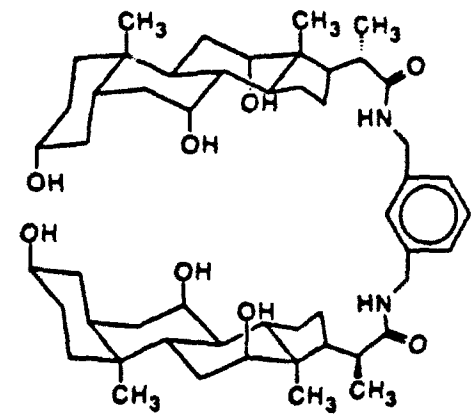
p-cholamide dimer



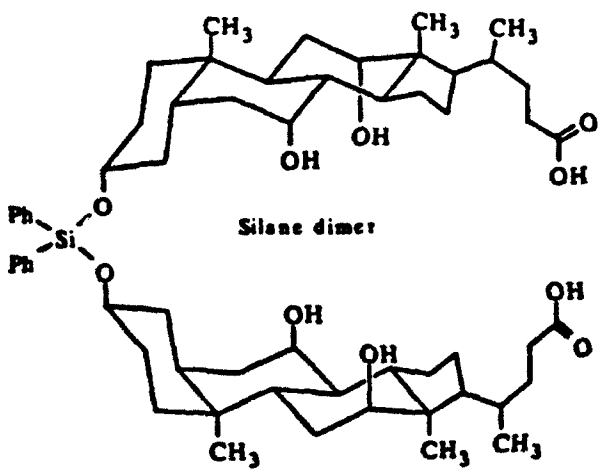
p-degradated cholamide dimer



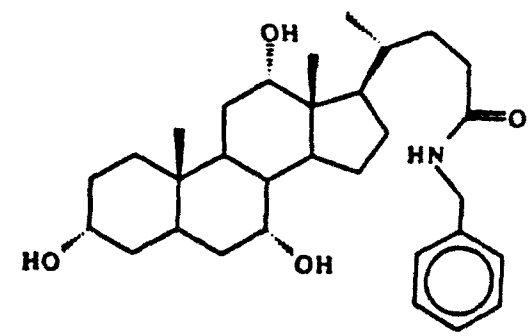
m-cholamide dimer



m-degradated cholamide dimer



Silane dimer



monocholamide

Linkage of the 3α-position with various spacers was investigated with the use of dichlorodiphenyl silane in order to form another type of clam-shaped dimer. Dichlorophenylsilane was chosen instead of dichlorophenylborane in order to avoid the formation of a tetra-coordinated borane salt. The silane dimer was made and used in NMR binding studies with amyl-glucoside. Unfortunately no chemical shifts changed for either the guest or host in the ^{1}H- and ^{13}C-NMR spectra. The results may be due to the loss of the silane dimer binding ability, since the most accessible 3α-position hydroxy group had been used as a linking point.

A literature procedure was used to reduce the carboxylic acid of cholic acid yielding the tetrol. The linkage of the primary alcohol with dichlorodiphenylsilane was attempted in order to form another dimer (Fig.14a), this would leave the 3α-hydroxy group available for binding. Unfortunately, 3α-hydroxy group (a secondary alcohol) competed with the primary hydroxy group for linkage with the spacer.

Figure.14a

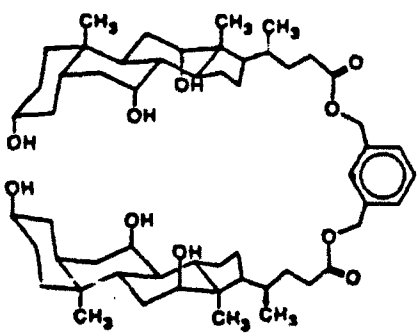
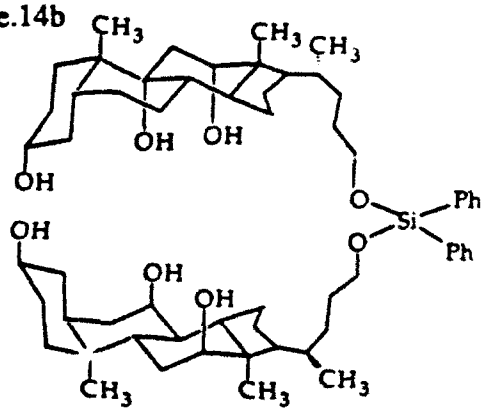


Figure.14b



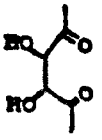
The twisted m-cholamide dimer may be caused by the intramolecular hydrogen bonding between two amides. To solve this problem, we tried to synthesize a m-xylylene cholate diester (Fig.14b). Because there is no hydrogen atom on an ester bond , there is no possibility of having intramolecular hydrogen bonding in the host, which may allow the two cholate arms of the dimer

to be accessible for binding.

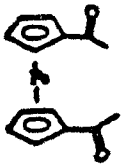
The synthesis was attempted using m-xylylenediol as a spacer which has two primary hydroxy groups for coupling with cholic acid. N,N'-carbonyldiimidazole was used as the activating reagent for the carboxylate of cholic acid. Unfortunately the secondary hydroxyl group of the 3α-position (the least hindered one) seemed to react with another molecule of cholic acid. We therefore tried to protect this hydroxyl group before running the coupling reaction. This synthesis is currently under investigation.

One possible way to solve the twisted structural problem of hosts is to use more rigid dimers to fix the clam shape conformations. The next step is to try the p- & m-bis-nor-cholamide dimers in binding studies with amyl glucoside by ^{1}H-NMR. The other way to solve the conformation problem is to make the macrocyclic hosts more rigid for binding with the glucoside. For example, the side chain ester of the silane dimer could be hydrolzyed to produce the free acid, which could then be linked with para- or meta-xylylenediamine. Hydrolysis in NaOH solution was used to remove the methoxy group to form the sodium salt, but the use of dilute HCl aqueous solution to produce a free acid caused the diphenylsilyl group to be removed. This may occur because the silyl group is not stable in acidic solution. Other spacers to be examined in the future are proposed below:

oxalated diester bridge



tartrate bridge



ferrocene bridge

III. Theoretical modelling:

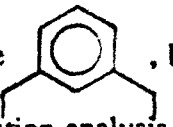
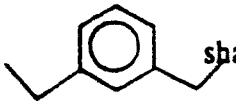
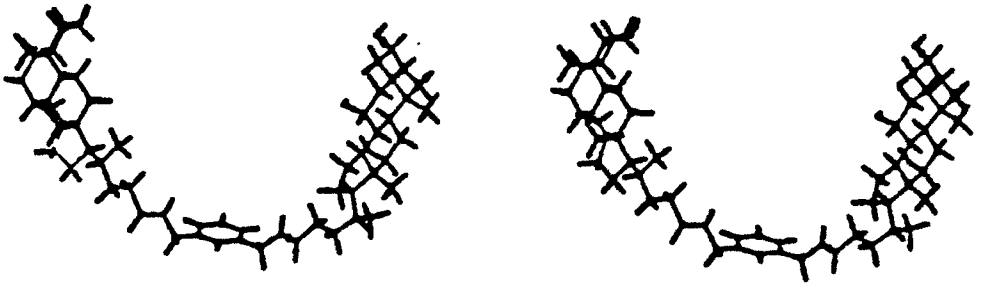
All molecular modeling has been performed by Ms. Suzanne M. Evans and Prof. Carol Venanzi(Ref.16). The molecular mechanics calculations on the para- and meta-cholamide dimers have been done by varying the angles of the side chain bonds or the distances of the two C-3 oxygens. In the m-cholamide dimer case, the results showed the designed clam-shape(C-shape) structures have energy at least 4.5-5 Kcal/mol above the global minima energy. The C-shaped dimer with a m-xylylene spacer did not make the cholic acid arms parallel like  , but  shaped, with CH_2 to CH_2 distance = 11.16Å (Fig.15). The conformation analysis by distance showed the distance between the two C3-oxygens was 14.78Å in the global minimum energy structure. Most of the favored conformations were twisted S-shape or helical structures which may be due to the intramolecular hydrogen bonding between two amides.

Figure.15



In the p-cholamide dimer case, the clam shape conformations were not as we had hoped, rather, they are cupped (with a side & a bottom) or twisted S-shapes. The cupped-shape conformation was similar to the shape found from arabinose binding protein. This suggested the p-dimer is a better molecule than m-dimer in binding sugars.

IV.Experimental section :

1. Cholate ester(Ref.17):

A stirred solution of cholic acid (0.825 g, 2 mmol) or triformyl bis-nor-cholic acid (0.93 g, 2 mmol) in tetrahydrofuran (THF, 20 mL) containing acetonitrile (5 mL) was cooled to 10°C under nitrogen. A solution of N-hydroxysuccinimide (0.45 g, 3.9 mmol) and dicyclohexyl-carbodiimide (DCC, 0.45 g, 2.2 mmol) in THF (5 mL) was then added to the acid solution by dropwise addition. Then the existing solution was stirred at room temperature overnight. After filtration, chloroform (60 mL) was added into the reaction solution. The organic layer was extracted with 10% $NaHCO_3$ (60 mL×2) and water (40 mL). Organic layer was then dried with $MgSO_4$. After filtration and evaporation, the white crude product was collected in 56% yield.

For the cholate ester: Rf=0.38 (25% benzene/acetone); Rf= 0.26 (10% $CH_3OH/CHCl_3$, silica gel). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.63 (3H s, 18-CH_3), 0.82 (3H s, 19-CH_3), 0.95 (3H d, 21-CH_3), 2.76 (2H s, $NCOCH_2$), 3.39 (1H m, 3β-CH), 3.78 (1H s, 7β-CH), 3.90 (1H s, 12β-CH) ppm. ^{13}C-NMR ($CDCl_3$, 300 MHz): δ 12.5, 17.2, 68.3, 71.9, 72.9, 169.0 ppm. FT-IR (KBr): 3100-3600 br (OH), 2800-3000 br(aliphatic-CH), 1813 m, 1783 m, 1737 (s, ester) cm^{-1}.

For the triformyl bis-nor-cholate ester: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.75 (3H s, 18-CH_3), 0.89 (3H s, 19-CH_3), 1.20 (3H d, 21-CH_3), 2.76 (2H s, $NCOCH_2$), 3.85 (1H m, 3β-CH), 5.01 (1H br s, 7β-CH), 5.17 (1H br s, 12β-CH), 8.03, 8.10, 8.12 (1H s, 3α,7α,12α-OCHO) ppm. ^{13}C-NMR ($CDCl_3$, 300 MHz) representataive peaks beyond 50 ppm: δ 70.4, 73.6, 74.5, 160.3, 160.4, 160.48, 160.53, 169.0, 170.9 ppm.

2. m-Cholamide dimer, p-cholamide dimer and mono-cholamide :

The activated cholate ester (1.36 g, 2.7 mmol) in N,N'-dimethylforamide (DMF, 30 mL)

was added m-xylylenediamine (0.18 mL, 1.35 mmol) or p-xylylenediamine (0.1849 g, 1.35 mmol) or benzylamine (0.15 mL, 1.35 mmol) and stirred at room temperature overnight. After evaporation of solvent, the residue was added into stirred diethyl ether to produce white precipitate. The dimer or monomer could be obtained in 60% yield by evaporating diethyl ether and a trace of DMF.

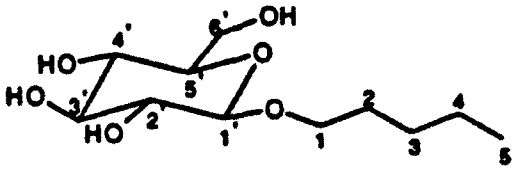
Characterization:

For the m-cholamide dimer : Rf= 0.66 (10% $CH_3OH/CHCl_3$, silica gel). ^{1}H-NMR (CD_3OD, 300 MHz): δ 0.61 (6H s, CH_3), 0.84 (6H s, CH_3), 0.94 (6H d, CH_3's), 3.37 (2H m, 3-CH), 3.72 (2H br s, 7-CH), 3.86 (2H br s , 12-CH), 4.52 (4H br, benzylic), 7.11-7.26 (4H m, aromatic). FT-IR (KBr): 3100-3600 br (OH), 2500-3000 br (aliphatic-CH), 1646 s (amide) cm^{-1}.

For the p-cholamide dimer : ^{1}H-NMR (CD_3OD, 300 MHz): δ 0.61 (6H s, 18-CH_3), 0.84 (6H s, 19-CH_3), 0.94 (6H d, 21-CH_3), 3.37 (2H m, 3β-CH), 3.72 (2H br s, 7β-CH), 3.86 (2H br s, 12β-CH), 4.25 (4H br, $ArCH_2$), 7.15 (4H s, aromatic) ppm.

For the mono-cholamide: ^{1}H-NMR (5% $CD_3OD/CDCl_3$, 300 MHz): δ 0.61 (3H s, 18-CH_3), 0.83 (3H s, 19-CH_3), 0.91 (3H d, 21-CH_3), 3.40 (1H m, 3β-CH), 3.77 (1H d, 7β-CH), 3.90 (1H s, 12β-CH), 4.03 m (2H s, OH & NH), 4.36 (2H d, benzylic), 7.19 & 7.23 (5H m, aromatic). FT-IR (KBr): 3100-3600 br (OH), 2500-3000 br (aliphatic-CH), 1670 s, 1625 s (amide) cm^{-1}.

3. ^{1}H- and ^{13}C-NMR chemical shift assignments of amyl glucoside (Ref.19):



The peaks were assigned by using a ^{1}H-^{13}C 2D-NMR spectrum at 250 MHz and a ^{1}H-^{1}H 2D-NMR spectrum at 600 MHz.

^{1}H-NMR ($CDCl_3$, 600 MHz) representative peaks: δ 0.96 (3H t, 5-CH_3), 1.38 (4H m, 3&4-CH_2), 1.69 (2H m, 2-CH_2), 3.37 (1H d, 5'-CH), 3.44 (1H t, 2'-CH), 3.59 (2H m, 1- CH & 3'-CH), 3.67 (1H t, 4'-CH), 3.91 (3H br m, (1-CH & 6'-CH_2), 4.31 (1H d, 1'-CH) ppm. ^{13}C-NMR ($CDCl_3$, 250 MHz): δ 14.1 (5-C), 22.6, 28.1 (3&4 -C), 29.4 (2-C), 61.7 (6'-C), 70.0 (5'-C), 70.6 (1-C), 73.6 (3'-C), 75.7 (2'-C), 76.5 (4'-C), 103.0 (1'-C) ppm.

4. Synthesis of diphenylsilyl cholate dimer (Ref.19):

Methyl cholate (0.85 g, 2 mmol) in of dry benzene (20 mL) containing triethylamine (0.28 mL, 1 mmol) was added to dichlorodiphenylsilane (0.21 mL, 1 mmol) at 0°C. After the solution was warmed to room temperature, white precipitate was formed, then the existing solution aws refluxed for three days. The solution was then cooled to room temperature, filtered to remove $Et_3N{\cdot}HCl$ and the filtrate was evaporated to yield a light yellow residue. After diethyl ether was added to the residue, the solution was extracted with water, and dried over $MgSO_4$, to yield a white precipitate (31% yield). Rf=0.72 (silical gel, MeOH:$CHCl_3$= 1:14). ^{1}H-NMR ($CDCl_3$, 300 MHz) : δ 0.61 (3H s, 18-CH_3), 0.76 (3H s, 19-CH_3), 0.92 (3H d, 21-CH_3), 3.57 (1H m, 3-CH), 3.60 (3H s, CO_2CH_3), 3.63 (1H br s, 7-CH), 3.91 (1H br s, 12-CH), 7.28 (3H m, aromatic), 7.57 (2H d, aromatic) ppm. ^{13}C-NMR ($CDCl_3$, 600 MHz) representative peaks beyond 60 ppm: 68.71, 73.17, 73.99, 127.78, 129.85, 134.50, 135.08, 174.80 ppm. FT-IR (KBr): 3100-3600 br (OH), 2700-3000 br (aliphatic), 1739 s (ester), 1174 s (O-Si) cm^{-1} . FAB-MS (in NBA, nitro benzyl alcohol): m/z = 1027.6, 1026.6, 1025.7(M^{+}).

5. Synthesis of 5β-cholane-3β,7β,12β,24-tetrol (Ref.20):

Cholic acid (1g, 2.4 mmol) was dissolved in 50 mL of dry tetrahydrofuran (THF) and then 1M BH_3•THF (14.7 mL, 15.4 mmol, 6 equivalences) was added by dropwise addition. The existing solution was stirred at room temperature until a borane ester gel formed. THF was added to the solution until the borane ester dissolved. The solution was then stirred overnight at room temperature. The reaction was quenched by adding 1:1 acetic acid/water until there was no further gas evolution, then stirred for an additional hour. Water was then added to dilute the solution and 1:1 THF/diethyl ether was used to separate the solution into two layers. THF/diethyl ether(30mL×2) was used to extracted the aqueous layer and the organic layers was combined. The organic layers were then extracted with brine(125 mL×2), 1.5N KOH(125 mL×2) and brine(125 mL×2) and dried over $MgSO_4$. After filtration to remove $MgSO_4$, the organic layer was evaporated to dryness yielding a white solid (89% yield). ^{1}H-NMR (CD_3OD, 600 MHz): δ 0.48 (3H s, 18-CH_3), 0.68 (3H s, 19-CH_3), 0.79 (3H d, 21-CH_3), 3.12 (1H m, 3-CH), 3.28 (2H br s, 24-CH_2), 3.56 (1H br s, 7-CH), 3.72 (1H br s, 12-CH) ppm. ^{13}C-NMR (CD_3OD, 600 MHz): δ 14.2, 19.2, 64,8, 70.3, 74.1, 75.3 ppm. FT-IR (KBr): 3100-3500 br (OH), 2500-3000 br (aliphatic), 1300-1500 br (C-O) cm^{-1}.

6. Synthesis of p-hexaformyl cholamide dimer:

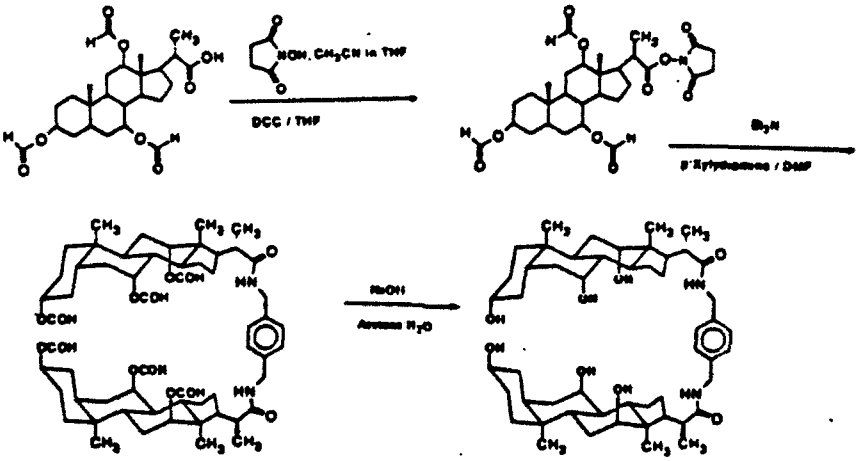
Nor-cholate N-hydroxysucciimide ester (0.51 g, 0.95 mmol) in dimethylforamide (DMF, 20 mL) was added to p-xylylenediamine (0.065 g, 0.48 mmol) solution (in 20 mL of DMF) at 0°C and the existing solution was stirred at room temperature overnight. After evaporation of the DMF, the light yellow solid was dissolved in $CHCl_3$, extracted with water and dried over $MgSO_4$, followed by filtration to remove $MgSO_4$ and evaporation of $CHCl_3$. The off-white solid was stirred in diethyl ether overnight. The dimer was obtained in 56% yield after evaporating diethyl

ether. ^{1}H-NMR (5% $CD_3OD/CDCl_3$, 300 MHz): δ 0.85 (6H s, 18-CH_3), 0.99 (6H s, 19-CH_3), 1.00 (6H d, 21-CH_3), 4.27 (4H dd, benzylic), 4.34 (2H m, 3-CH), 4.95 (2H br s, 7-CH), 5.11 (2H br s, 12-CH), 7.10 (4H d, aromatic), 7.92, 7.99, 8.09 (6H s, 3,7,12α-OCHO) ppm. FT-IR (KBr): 2600-3000 br (aliphatic), 1717 m (ester), 1670 s (amide), 1216 s (C-O) cm^{-1}.

7. Hydrolysis of p-hexaformyl cholamide dimer to p-bis-nor-cholamide dimer (Ref.21):

To a stirred solution of protected dimer (0.3 g, 0.3 mmol) in acetone (10 mL) was added 0.2 N NaOH (20 mL) dropwise over a peroid of 0.5 hour. The solution was then stirred overnight at room temperature. Then, 0.2 N acetic acid (20 mL) was added to acidify the solution. The precipitate was collected after evaporation of solvents. The solid was stirred with diethyl ether overnight. The product was obtained in 91% yield by evaporation of the diethyl ether. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.63 (6H s, 18-CH_3), 0.83 (6H s, 19-CH_3), 1.03 (6H d, 21-CH_3), 3.38 (2H m, 3-CH), 3.72 (2H br s, 7-CH), 3.83 (2H br s, 12-CH), 4.20, 4.29 (4H dd, benzylic), 7.55 s (4H d, aromatic) ppm. ^{13}C-NMR ($CDCl_3$, 300 MHz) peaks beyond 60 ppm: δ 67.8, 71.8, 72.5, 127.5, 137.1, 178.0 ppm. FT-IR (KBr): 3100-3500 br (OH), 2800-3000 br (aliphatic), 1670 s (amide) cm^{-1}.

Synthetic scheme for p-bis-nor-chlamide dimer:



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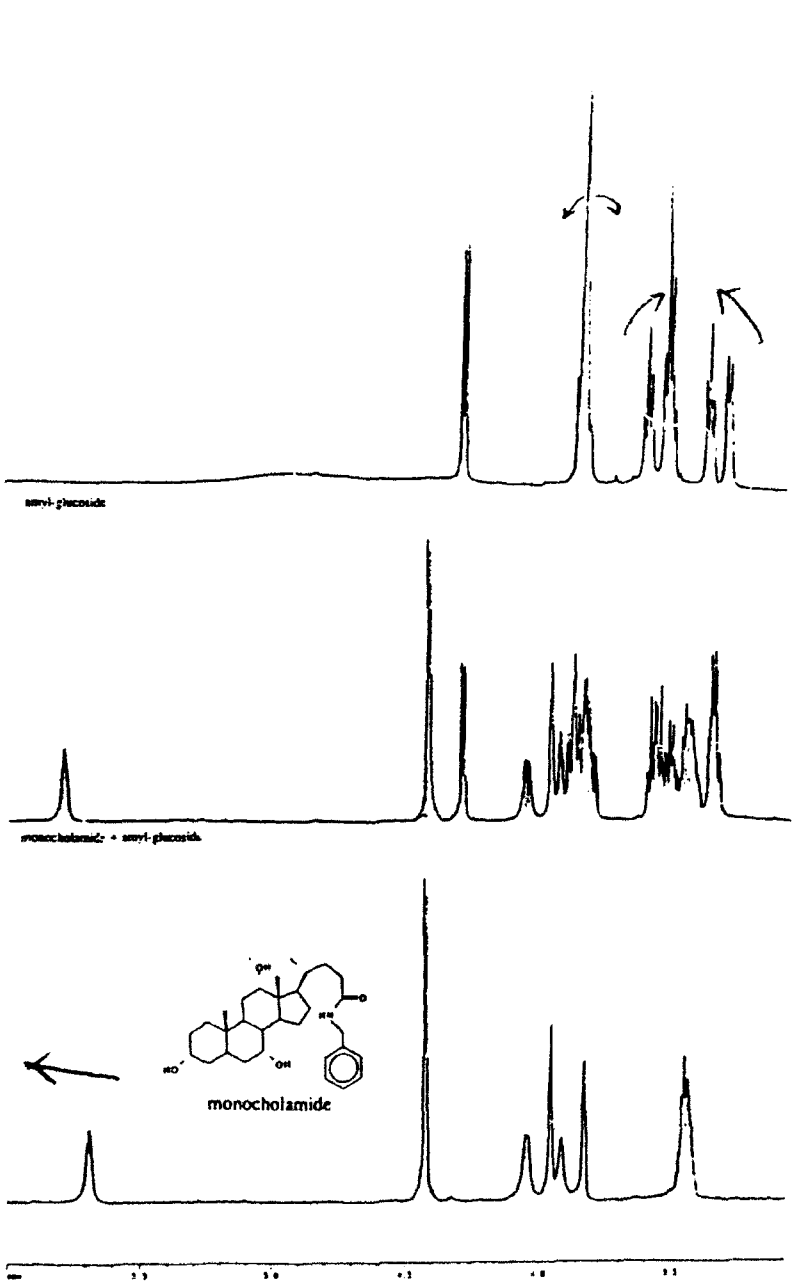
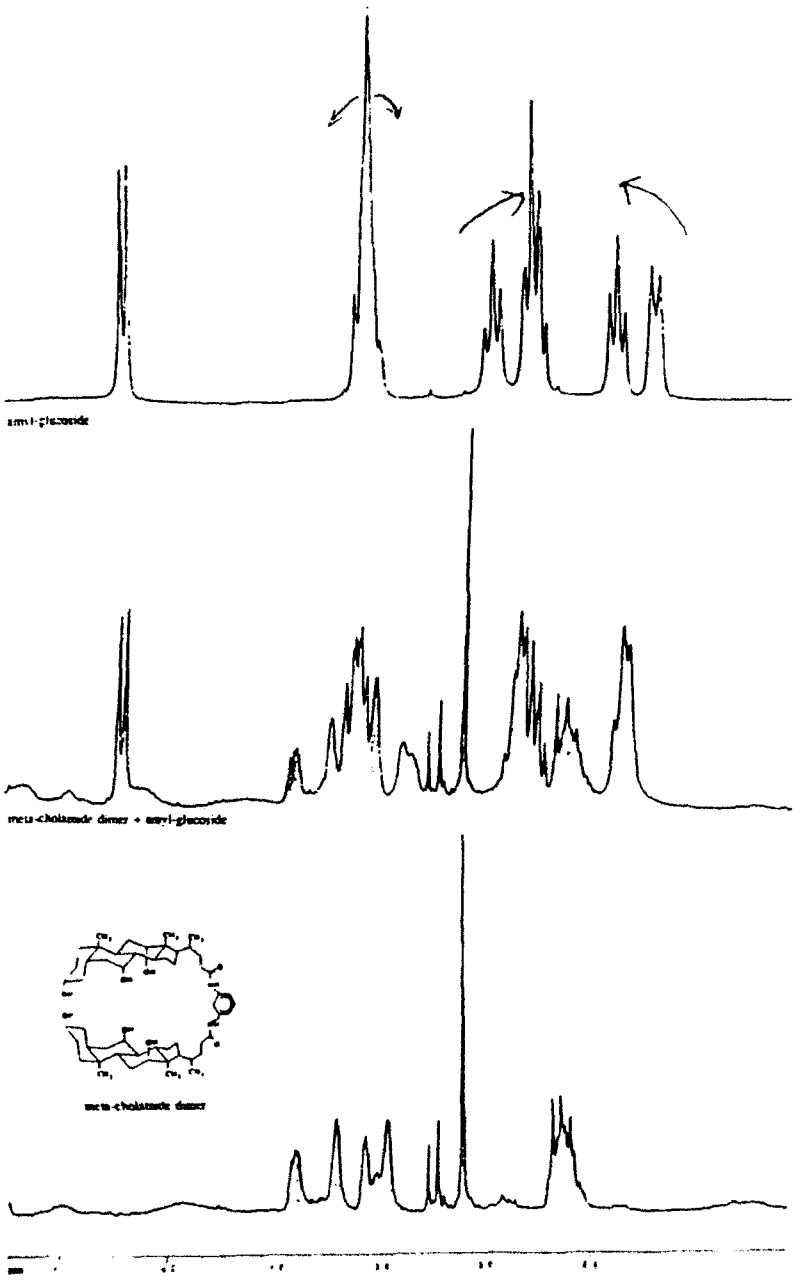
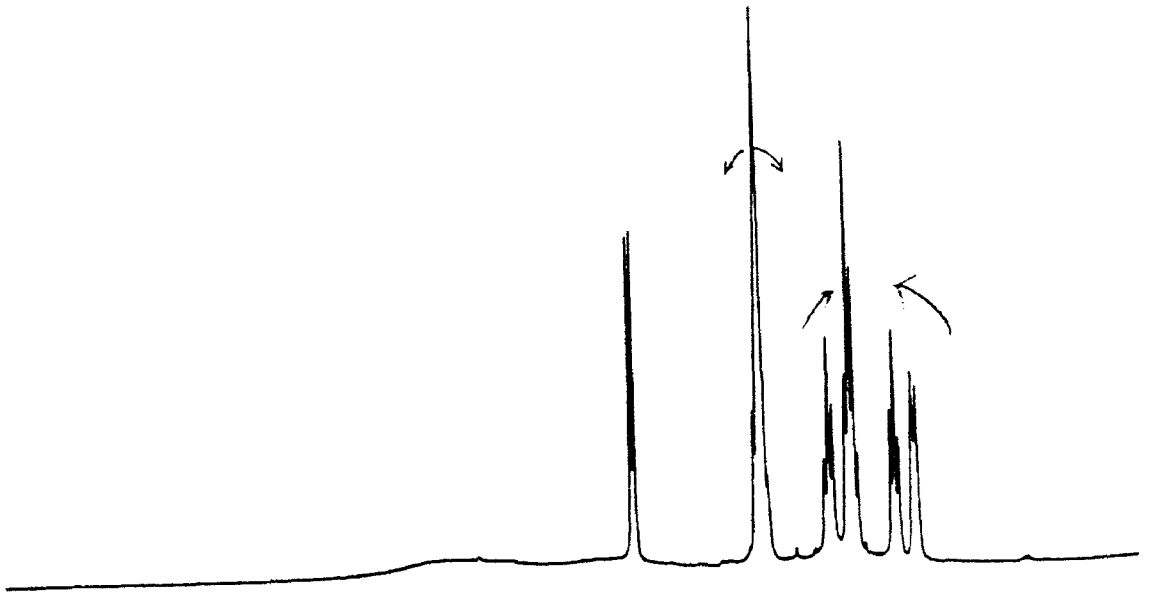
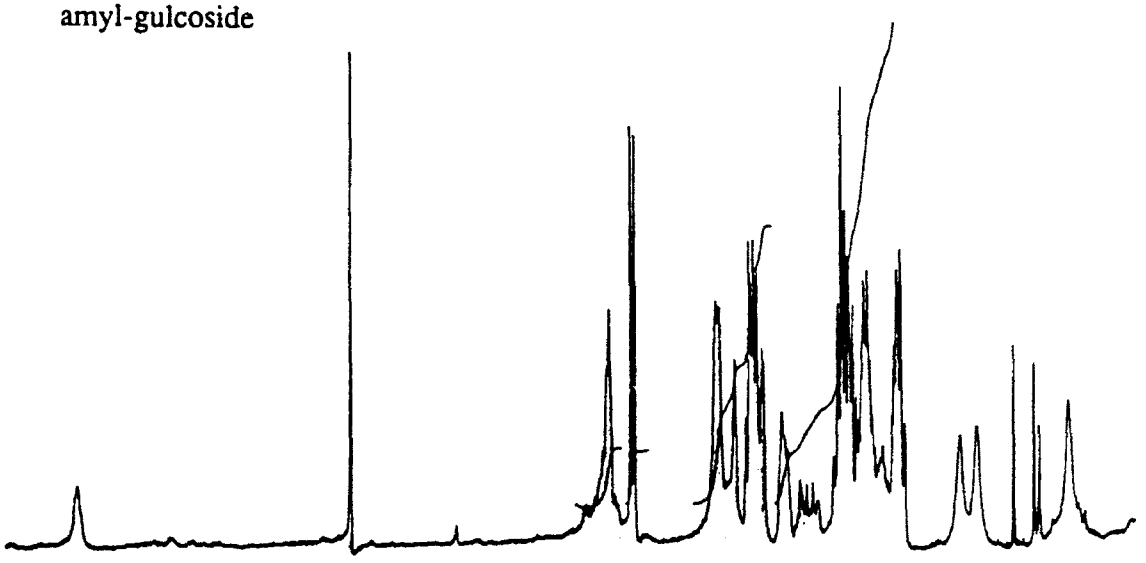
NMR Binding studies:







amyl-gulcoside



amylglucoside+p-cholamide dimer